

Table 1: HEPARANASE SEQUENCE HOMOLOGY DATA

	10	20	30	40	50	60
mouse	-MLR-----	L L L L W I W G P I G A L A Q G A P A G T A P T D D V V D L E F Y T K R P L R S V S P S F L S I T				
rat	-MLRP-----	L L L L W I W G R I R A L T Q G T P A G T A P T K D V V D L E F Y T K R L F Q S V S P S F L S I T				
human	MLLRSPALPPPLMLLLIGPLGELSPGALPRPAQAQDVVDLDFFTQEPHLVSPSFLSVT					
chicken	MLVLLLVLLAVPP-----	RR-TAEQLGLREPIGAVSPAFLSLT				
	*: * * *	*: * * *				
	70	80	90	100	110	120
mouse	IDASLATDPRFLTFLGS	PRLRALARGLSPAYLRFGGKTDFLI	FDPKKEPTS	EERSYWK		
rat	IDASLATDPRFLTFLGS	PRLRALARGLSPAYLRFGGKTDFLI	FDPKKEPTS	EERSYWK		
human	IDANLATDPRFLILIGSPKLR	TLARGLSPAYLRFGGKTDFLI	FDPKKEPTS	EERSYWK		
chicken	LDASLARDPRFVALLRH	PKLHTLASGLSPGLRFGGKTDFLI	FNPKDSTWEKVLSEF			
	: * * * * *	: * * * * *	: * * * * *	: * * * * *	: * * * * *	: * * * * *
	130	140	150	160	170	180
mouse	QVNNDICRSEFVSAAVL	RKLQVEWPFQELLLR	EQYQKEFNSTY	SRSSVDM	LYSFAKCS	
rat	QDNNDICGSEVSADVL	RKLQMEWPFQELLLR	EQYQREFKNSTY	SRSSVDM	LYSFAKCS	
human	QVNQDICKYGSIPDVE	RKLRLWPFQELLLR	EHYQKFNSTY	SRSSVDV	LYTFANC	
chicken	QAK-DVCEAWPSFAVVP	KLTLQWFLQEKLLA	ESWKKHNTT	TRSTLDI	LHTFASS	
	*: * * *	: * * * *	: * * * *	: * * * *	: * * * *	: * * * *
	190	200	210	220	230	240
mouse	GLDLIFGLNALLRTP	PDLRWSSNAQLLLDY	CSSKGYNISWELGNE	PNSFWKKAH	ILIDGL	
rat	RLDLIFGLNALLRTP	PDLRWSSNAQLLLDY	CSSKGYNISWELGNE	PNSFWKKAH	ISIDGL	
human	GLDLIFGLNALLRTP	ADLQWSSNAQLLLDY	CSSKGYNISWELGNE	PNSFLKAD	IFINGS	
chicken	GFRLVFGNALLRRAGL	QWSSNAQLLLGYCAQ	RSYNISWELGNE	PNSFRKKS	GICIDGF	
	: * * * * *	: * * * * *	: * * * * *	: * * * * *	: * * * * *	: * * * * *
	250	260	270	280	290	300
mouse	QLGEDFVELHKL	QRS-AFQNAKLYGPDIG	QPRGKTVKLLRS	SFLKAGGEV	IDSLTWH	HHYY
rat	QLGEDFVELHKL	QKS-AFQNAKLYGPDIG	QPRGKTVKLLRS	SFLKAGGEV	IDSLTWH	HHYY
human	QLGEDYIQLHKL	LRKS-TFQNAKLYGPDVG	QPRKTKAKMLK	SFLKAGGEV	IDSVTWH	HHYY
chicken	QLGRDFVHLRQL	LSQPLYPHAEYGLDVG	QPRKHTQHLRS	FMKSGGKA	IDSVTWH	HHYY
	: * * * * *	: * * * * *	: * * * * *	: * * * * *	: * * * * *	: * * * * *
	310	320	330	340	350	360
mouse	LNGRIATKEDFLSSDALDT	FILSVQKILKVTKEIT	PGKKVWLGETSSAYGGG	APL	LSNTF	
rat	LNGRVATKEDFLSSDVLDT	FILSVQKILKVTKEIT	PGKKVWLGETSSAYGGG	APL	LSNTF	
human	LNGRTATREDFLNPDVLDI	FISSVQKVQVVESTR	PGKKVWLGETSSAYGGG	APL	LSDTF	
chicken	VNGRSATREDFLSPEVLDS	FATAIHDLGIVEATV	PGKKVWLGETSSAYGGG	APL	LSNTY	
	: * * * * *	: * * * * *	: * * * * *	: * * * * *	: * * * * *	: * * * * *
	370	380	390	400	410	420
mouse	AAGFMWLDKLGLSAQMG	IEVVMRQVFGAGNYHLVDEN	FEPLPDYWL	SLLFKKL	LVGPRVL	
rat	AAGFMWLDKLGLSAQMG	IEVVMRQVFGAGNYHLVDEN	FEPLPDYWL	SLLFKKL	LVGPKVL	
human	AAGFMWLDKLGLSARMG	IEVVMRQVFGAGNYHLVDEN	FDPLPDYWL	SLLFKKL	LVGTVKL	
chicken	VAGFMWLDKLGLAARRG	IDVVMRQVFGAGSYHLVDAG	FKPLPDYWL	SLLYKRL	LVGTRVL	
	: * * * * *	: * * * * *	: * * * * *	: * * * * *	: * * * * *	: * * * * *
	430	440	450	460	470	480
mouse	LSRVKGPDRSKLRVYL	HCTNYHPRYQEGDL	TLVNLHNVTKH	LKVPPL	FRKPVD	TYL
rat	MSRVKGPDRSKLRVYL	HCTNYHPRYREGDL	TLVNLHNVTKH	LKLP	PPMFSR	PDYKL
human	MASVQGSRRKRLRVYL	HCTNIDNPRYQEGDL	TLVNLHNVTKY	LRLPYP	FSNKQV	DKYL
chicken	QASVEQADARRLRVYL	HCTNPRHPKYREGDVT	LALNLSNVTQS	LQLPKQL	WSKS	VDQYL
	: * * * * *	: * * * * *	: * * * * *	: * * * * *	: * * * * *	: * * * * *
	490	500	510	520	530	540
mouse	LKPSGPDGLLSKSVQ	LNGQILKMDVDEQTL	PAITEKPLPAGS	SALSLPAF	SYGFFVIR	NAKI
rat	LKPFSGDGLLSKSVQ	LNGQILKMDVDEQTL	PAITEKPLPAGS	SSLVPAF	SYGFFVIR	NAKI
human	LRPLGPHGLLSKSVQ	LNGQLTKMDVDDQTL	PPIMEKPLRPGSS	LGLPAF	SYSFVIR	NAKV
chicken	LLPHGKDSILSREVQ	LNGRLQMDVDETL	PALHEMALPGST	LGLPAF	SYGFFVIR	NAKA
	: * * * * *	: * * * * *	: * * * * *	: * * * * *	: * * * * *	: * * * * *
mouse	AACI (SEQ ID NO:2)					
rat	AACI (SEQ ID NO:3)					
human	AACI (SEQ ID NO:4)					
chicken	IACI (SEQ ID NO:5)					

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Table 2- FUNCTIONAL PEPTIDE EPITOPES OF HEPARANASE

Peptide	Amino acid sequence	Location in SEQ ID NO 10	Property
p8 #7 SEQ ID NO:7	PAYLRFGGTKTDFLIFDP K	89-107	C-terminus of P8 - Dimerization
pep38 SEQ ID NO:6	CTNTDNPRYK	437-446	located 5 amino acids downstream of a heparin binding site
pep8 SEQ ID NO:8	SWELGNEPNSFLKKA	219-233	contains the proton donor residue of the heparanase active site
pep9 SEQ ID NO:9	RPGKKVWLGETSSAY	334-348	contains the nucleophilic residue of the active site
Pep10 SEQ ID NO:10	TWHHYLNGRTATR	294-307	Designed according to a 3D model as a surface exposed sequence, which bridges substrate binding and active site.

*NOTE: Specific peptide sequences are underlined in the sequence below

*NOTE: Mature heparanase dimer sequences in **bold face**:

MLLRSKPALPPPLMLLLLGLPLSPGALPRPAQAQDVVDLDDFFTQEPLH
LVSPSFLSVTIDANLATDPRFLILLGSPKLRTLARGLSPAYLRFGGTKTD
FLIFDPKKESTFEERSYWQSQVNQDICKYGSIPPDVEEKLRLWPYQEQL
 LLREHYQKKFKNSTYSRSSVDVLYTFANCGLDLIFGLNALLRTADLQWN
 SSNAQLLLDYCSSKGYNISWELGNEPNSFLKKADIFINGSQLGEDFIQLH
 KLLRKSTFKNAKLYGPDVGQPRRKTA~~MLKSFLKAGGEVIDSV~~TWHHYL
 NGRTATREDFLNPDVLDIFISSVQKVFQVVESTRPGKKVWLGETSSAYGG
 GAPLLSDTFAAGFMWLDKLGLSARMGIEVVMRQVFFGAGNYHLVDENFDP
 LPDYWLSLLFKKLVGTKVLMASVQGSKRRKLRVYLHCTNTDNPRYKEGDL
 TLYAINLHNVTKYLRLPYPFSNKQVDKYLLRPLGPHGLLSKSVQLNGLTL
 KVVDDQTLPPIMEKPLRPGSSGLPAFSYSFFVIRNAKVAACI (SEQ ID NO:4)

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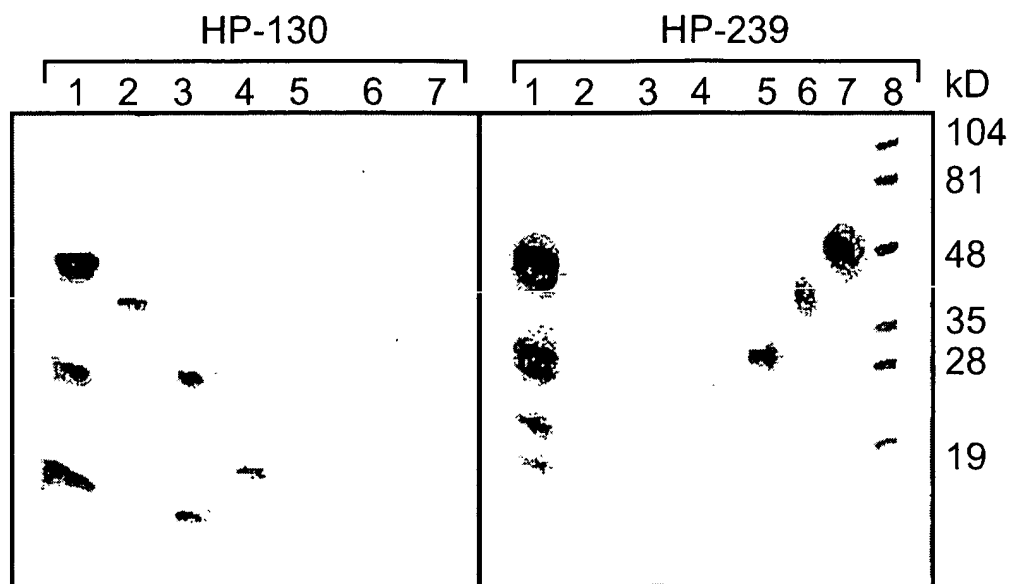


Fig. 1

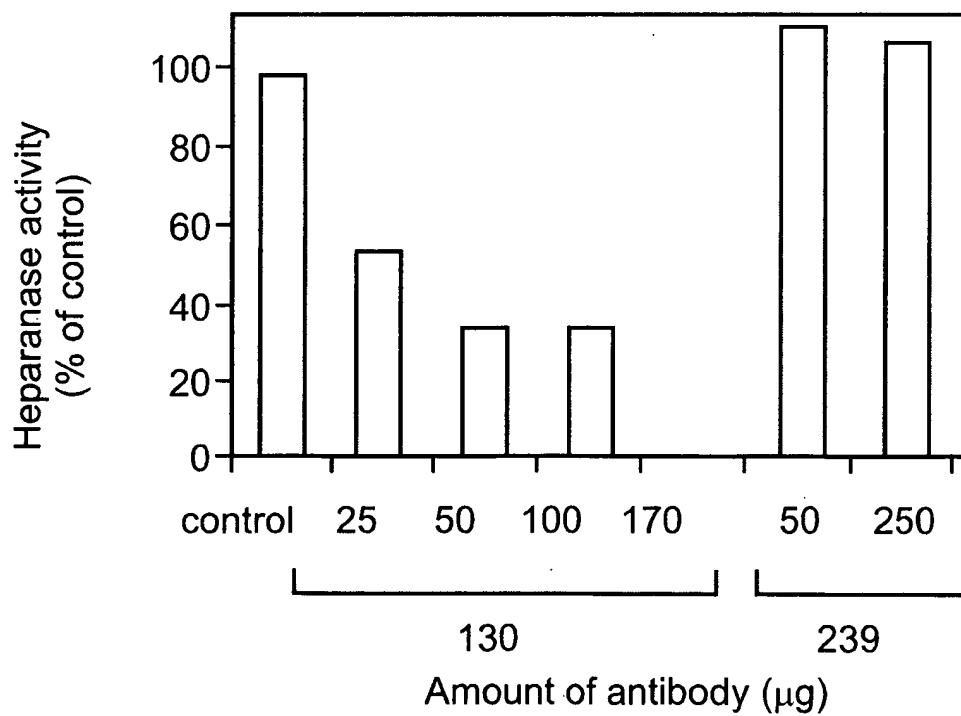


Fig. 2

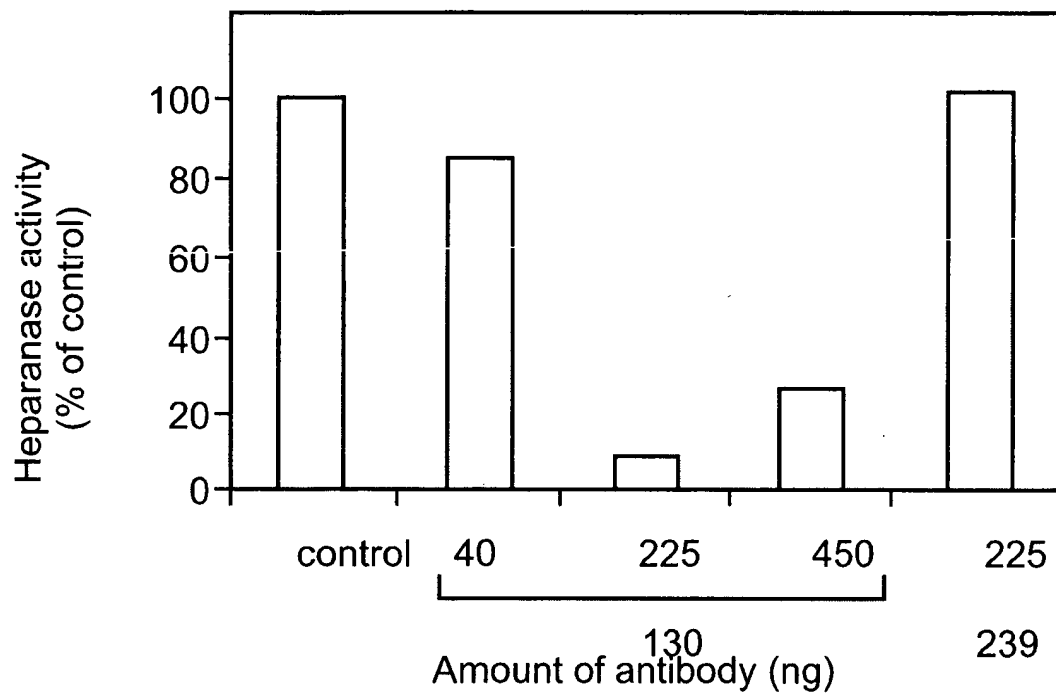


Fig. 3

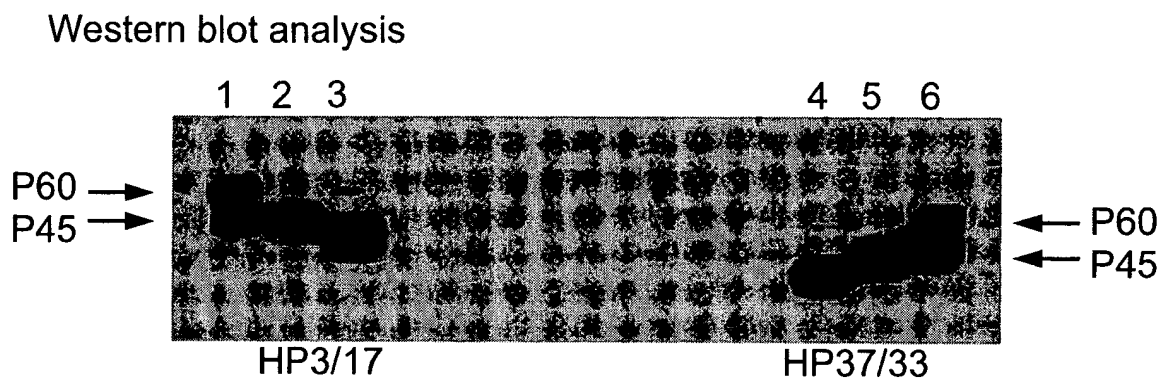


Fig. 4

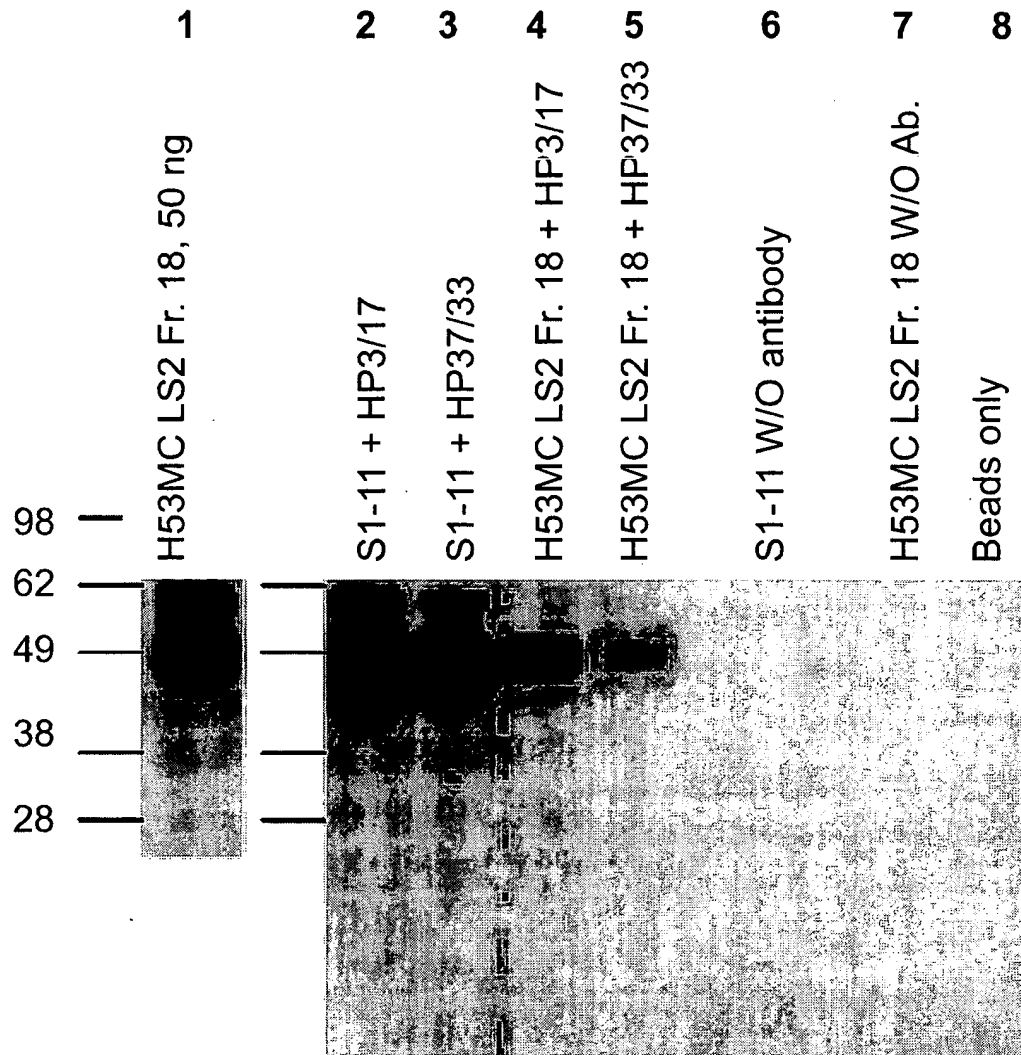


Fig. 5

Fig. 6a



Fig. 6c

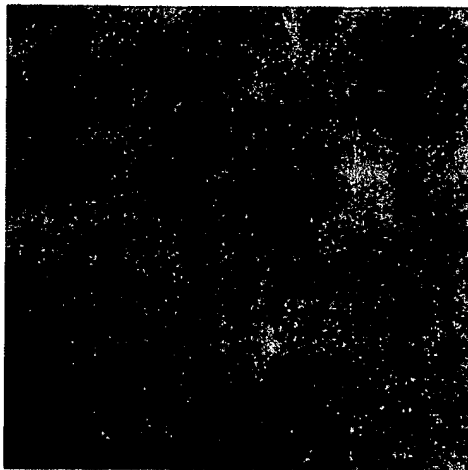
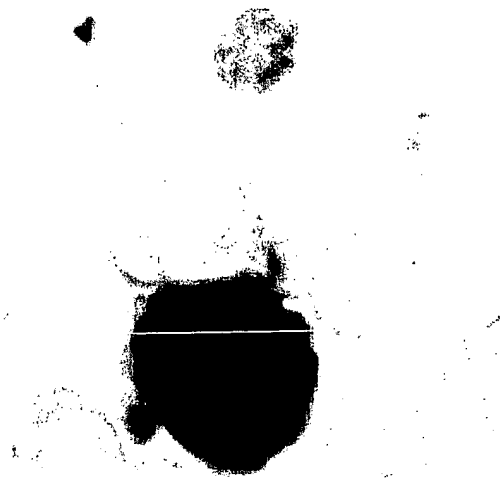
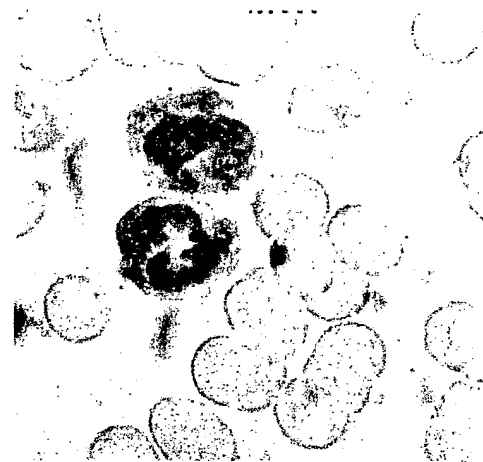


Fig. 7a

Fig. 6b



x 1000



HP37/33

Fig. 6d

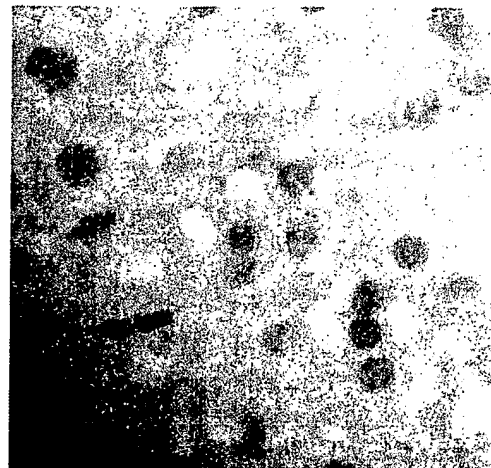


Fig. 7b

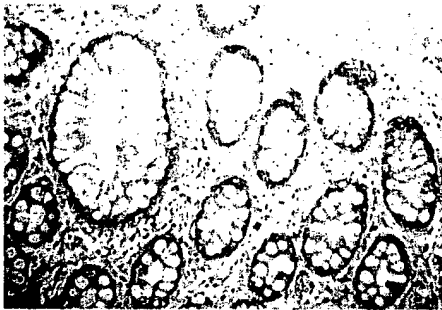


Fig. 8a



Fig. 8b

x 100



x 400

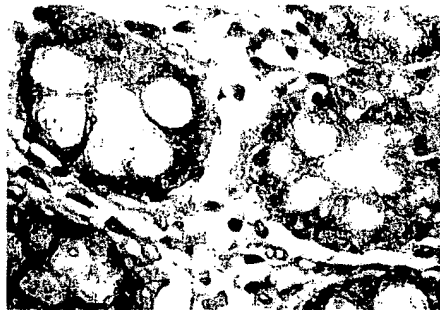


Fig. 9a

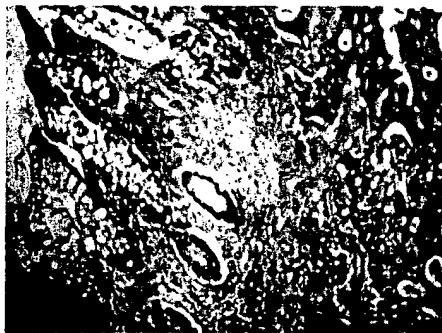


Fig. 9b

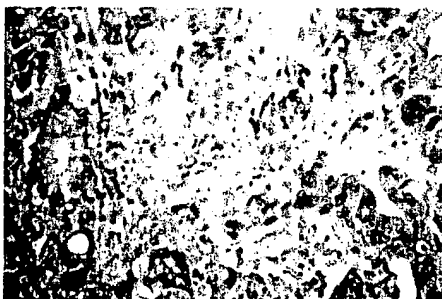


Fig. 9c

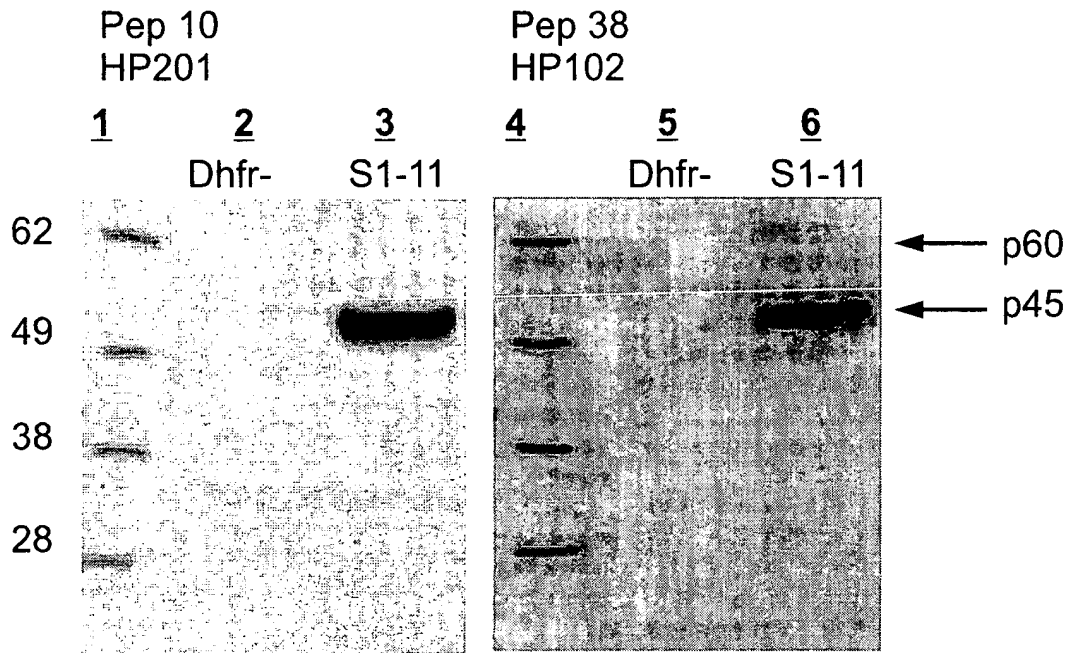


Fig. 10

Results

Group		Day 8	Day 12	Day 15	Day 18
A PBS	Mean	7.8	90.2	375.4	613.6
	SD	12.3	97.7	476.2	859.2
B HP130	Mean	0.0	34.2	188.4	298.2
	SD	0.0	42.8	165.6	111.7
C HP37/33	Mean	0.0	25.3	157.5	229.3
	SD	0.0	29.6	131.2	151.3

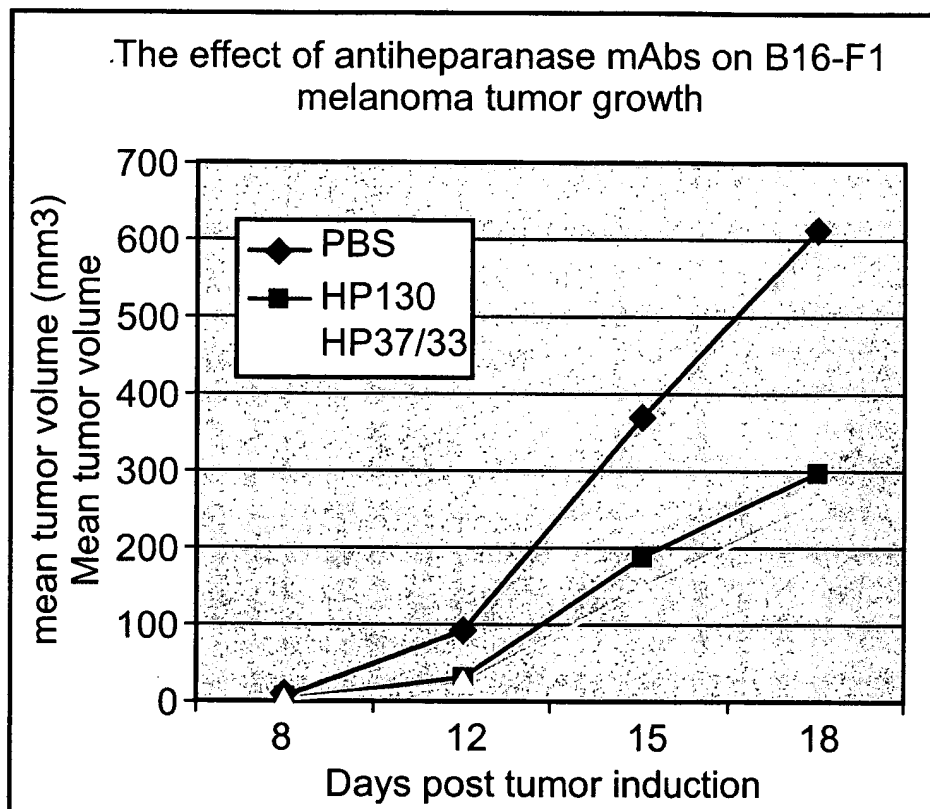


Fig. 11

Group	MEAN ARTHRITIC SCORE (4 legs)		
	Day 7	Day 11	Day 14
A-PBS	4.8	2.4	2.1
B-Mouse a human IgG3	4.9	3.4	2.5
C-HP3/17	4.2	2.1	1.5

Fig. 12

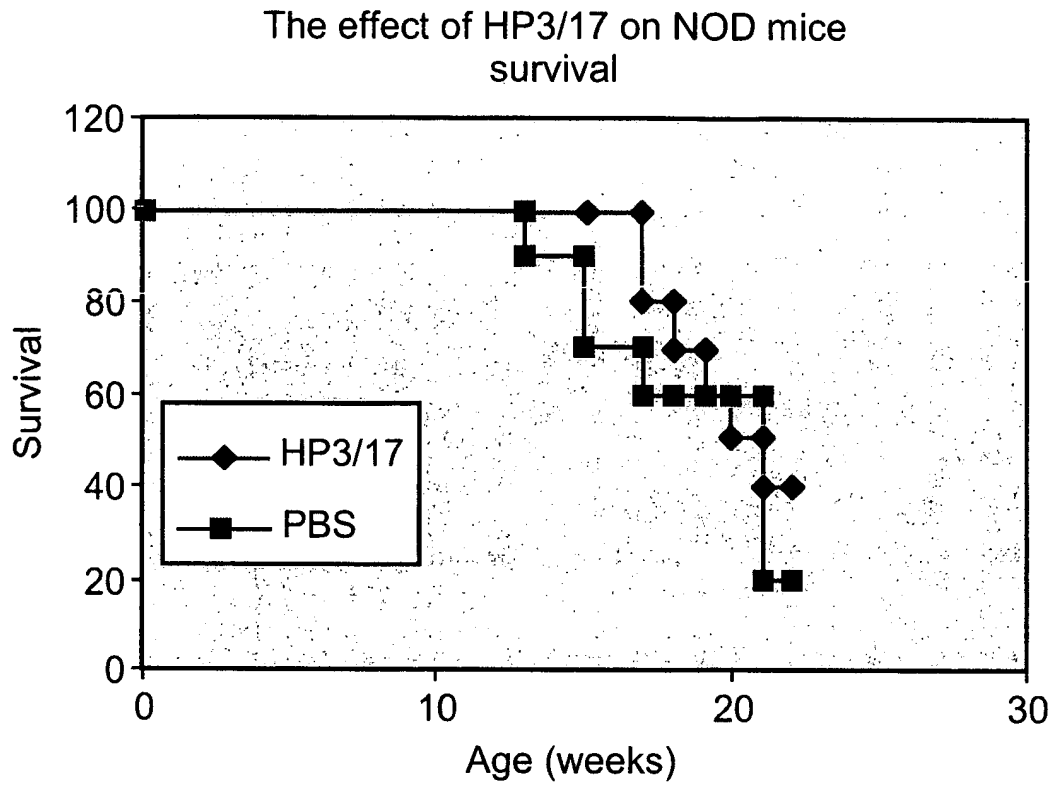


Fig. 13

HP37/33

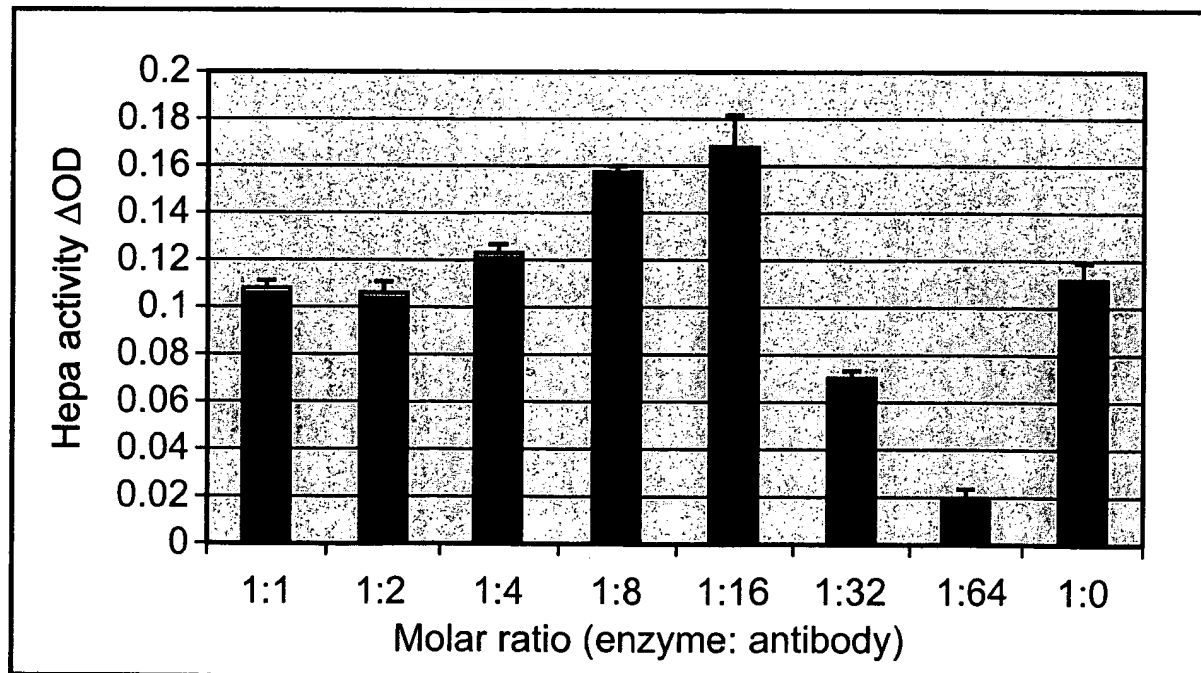


Fig. 14a

HP3/17

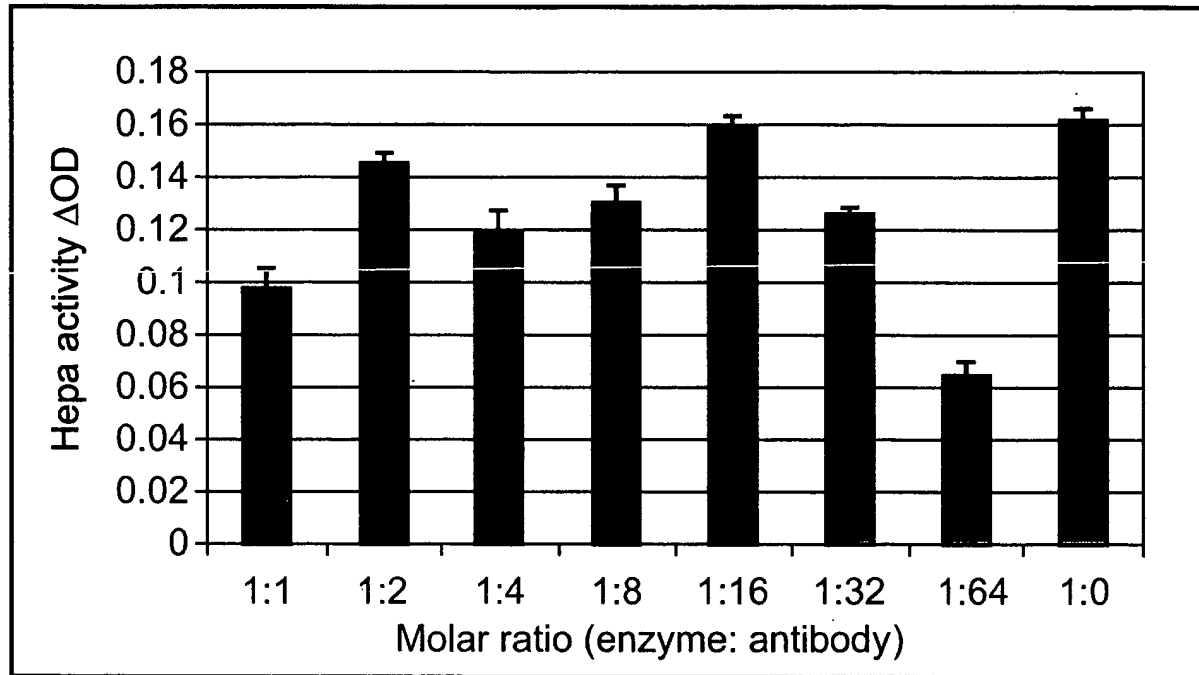
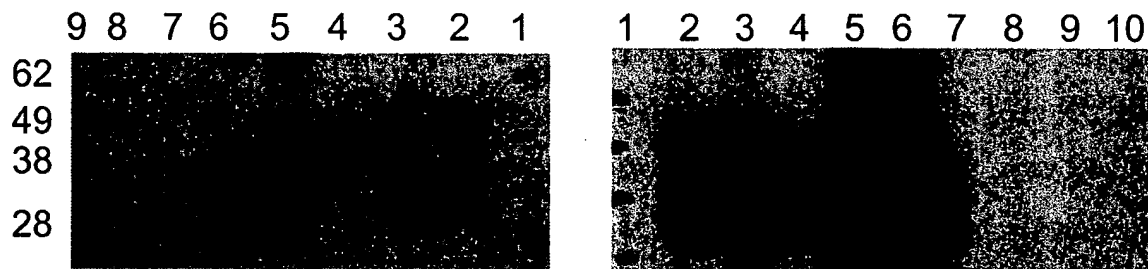


Fig. 14b

Epitope mapping

HP135.108

Fig. 15a

HP37/33

Fig. 15b

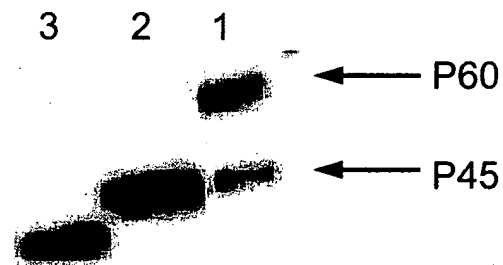
Western blot analysis

Fig. 16

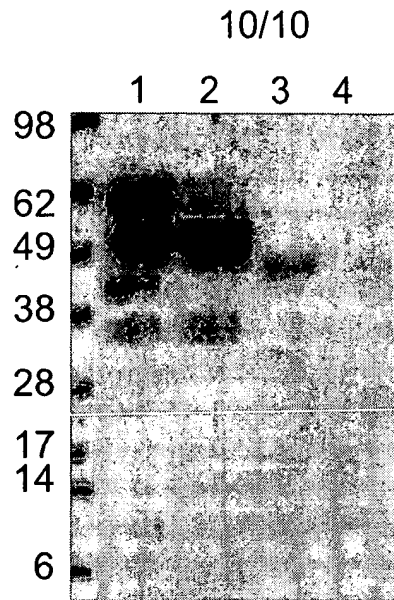


Fig. 17a

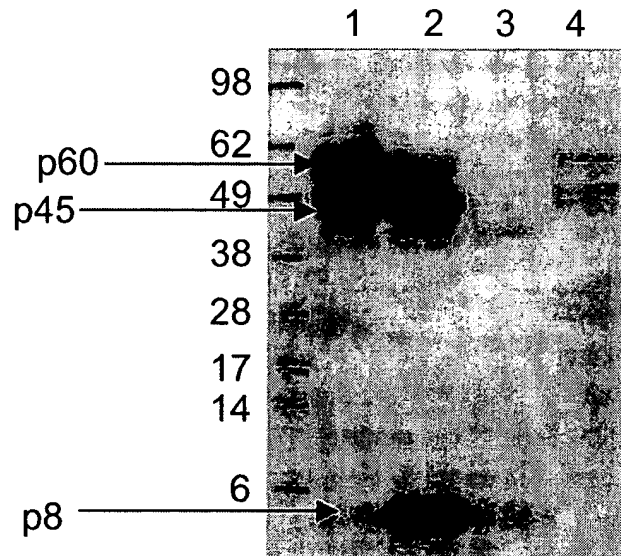


Fig. 17b

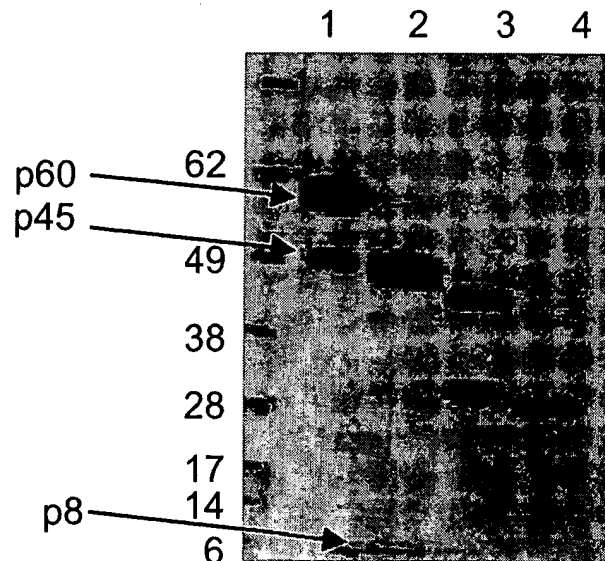


Fig. 17c